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Microfluidic PCR on Gold Nanorods for Combined Screening and Detection of ctDNA

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by

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Abstract

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Microfluidics and liquid biopsy on chip are growing in popularity as a method of detection and analysis used in point of care devices. The advantages of liquid biopsy include minimal invasiveness, low cost, shorter time from analysis to results, and less consumption of sample and reagents. Liquid biopsy has shown the ability to detect small concentrations of a biomarker of interest. However, some biomarkers, such as circulating tumor deoxyribose nucleic acid (DNA), exist in very small concentrations that make detection challenging. The integration of miniaturized polymerase chain reaction (PCR) with liquid biopsy techniques is a promising solution. Gold nanoparticles are used in some liquid biopsy techniques for sample capture and detection, and also function as light-to-heat converters that are useful in thermal cycling for PCR.

The capabilities of both lasers and light-emitting diodes (LEDs) to heat solutions of gold nanoparticles were tested. While the laser only led to minimal heating, the LED demonstrated promising results, achieving an 11°C temperature increase after 1 minute. LED-powered PCR is also more suitable for point of care applications, because of its low cost and ease-of-use. While there is significant research with regards to laser-powered heating, the research on LED-powered heating is significantly lacking in comparison. There is a need to further investigate and optimize LED-powered heating, with regards to nanoparticle shape and concentration, LED wavelength and voltage input, and thermal cycling temperatures.

Acknowledgements

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Table of Contents

List of Tables

List of Figures

Introduction

Recent research has shown the utility of gold nanoparticles in aiding thermal cycling for rapid, miniaturized PCR. When irradiated with a light source, the nanoparticles convert the light energy to thermal energy that heats the surrounding solution. Thus, pulses of light energy have the ability to drive the temperature cycles necessary for PCR. Existing research is primarily focused on lasers as an excitation source. However, LEDs have also been shown to effectively power PCR, and they may be more applicable to low-cost, point-of-care devices and techniques.

The following work investigates the use of LEDs to heat solutions containing gold nanoparticles, towards the goal of integrating miniature PCR with detection by liquid biopsy. The paper first provides a literature review of PCR and its miniaturization, heating of gold nanorods, and previous research conducted using gold nanorods. Next, the research design and protocols will be presented, followed by results. Finally, applications of this research to low-cost PCR and to rapid diagnostics will be briefly discussed.

Literature Review

1.1 PCR Overview

The amplification of nucleic acids is often necessary for quantification and analysis. Samples of interest often contain a small amount of DNA of interest, which must be amplified for analyses such as sequencing, gel electrophoresis, or cloning. There currently exist several methods of amplification, including polymerase chain reaction (PCR), strand-displacement amplification (SDA), nucleic acid sequence-based amplification (NASBA), and rolling-circle amplification (RCA). PCR has been the most commonly used method, because of its relative accuracy, sensitivity, and simplicity.[1–3]

The reagents in a PCR reaction mixture are *Taq* polymerase, primers, template DNA, and nucleotides. Primers are short sequences of DNA that are complementary and anneal to single-stranded target DNA, flanking the region of interest to be amplified. Mixing the aforementioned components in a tube, and subjecting it to several cycles of heating and cooling leads to the synthesis of the desired DNA segment. PCR thermal cycles consist of the following steps: (1) Denaturation at 95°C, (2) Annealing at 50 – 65°C, and (3) Extension at 72 – 77°C. During Denaturation, the double-stranded DNA is separated into single strands that serve as a template for amplification. Annealing cools the reaction to allow primers to bind complementarily to the single-stranded DNA. Extension raises the temperature for DNA synthesis by *Taq* polymerase. The number of copies of the segment of interest is doubled after each cycle and 20 to 40 cycles are typically run in a PCR reaction. Conventional PCR uses a metal heating block

powered by Peltier elements to generate the heating and cooling cycles. The reaction takes 2 to 4 hours in total due to slow heat-transfer rates between the heating blocks and plastic PCR tubes.[4,5] If the heating could be done faster, this process would be rate limited by the enzymatic binding and could be done on the order of minutes rather than hours.

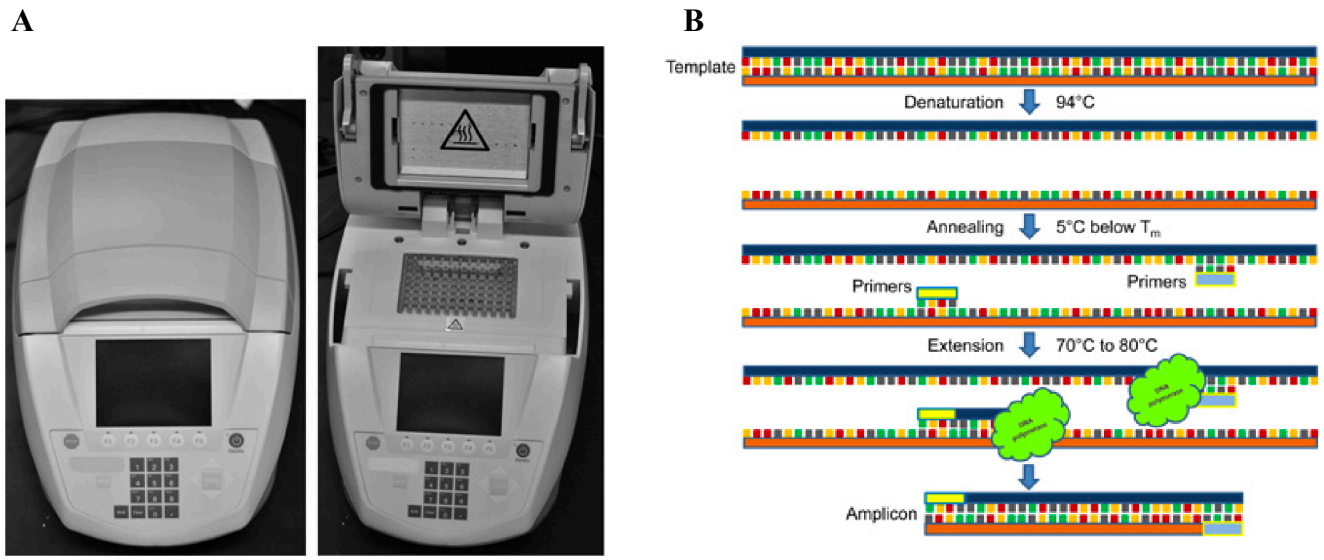

Figure 1. Traditional PCR instrument and process. (A) Traditional Peltier-based thermal cycler, shown closed on the left, and open with PCR tubes placed in the heating block on the right. (B) A standard PCR cycle consisting of denaturation of the DNA template, annealing of primers, and extension of the target DNA, yielding the amplicon.[5]

The miniaturization of PCR on chip may improve upon conventional PCR in terms of increased thermal cycling rates, as well as lower reagent consumption and short assay time. These advantages may make PCR on chip suitable for point-of-care (POC) diagnostics.[3]

1.2 PCR On Chip

Recent research has shown the possibility of PCR performed on microchip, allowing for fast and small-volume DNA amplification and analysis on a single chip. PCR on chip systems can vary in material, architecture, reaction volume, reaction speed, and heating method. Common materials include silicon, glass, and polymers such as polydimethylsiloxane, polycarbonate, and polymethylmethacrylate. The two major types of chip architectures are the stationary chamber, in which the PCR mixture is stationary and the reaction chamber cycles temperatures, and the continuous flow-through, in which the PCR mixture is pumped through a microfluidic channel. Advantages of continuous flow-through methods over stationary chambers include the potential for high throughput, potential for integration of PCR with analysis, and high speed limited only by the rate of synthesis by DNA polymerase. Challenges to PCR on chip remain, such as high cost, integration of a complete 'lab-on-a-chip', and detection of products.[2,3] A few developments in PCR on chip are highlighted below.

Kopp et al. reported a high-speed continuous-flow PCR performed on a glass microchip. The design and setup is shown in Figure 2. Three zones of the chip are kept at constant temperatures of 95°C, 77°C, and 60°C and PCR mixture is pumped through a channel that runs through the zones a defined number of times. The total flow-through time for 20 cycles ranges between 18.7 minutes and 90 seconds, with increasing flow rates and decreasing product quantity.[4] The drawback of such a continuous-flow system is the need for a external pump.[6]

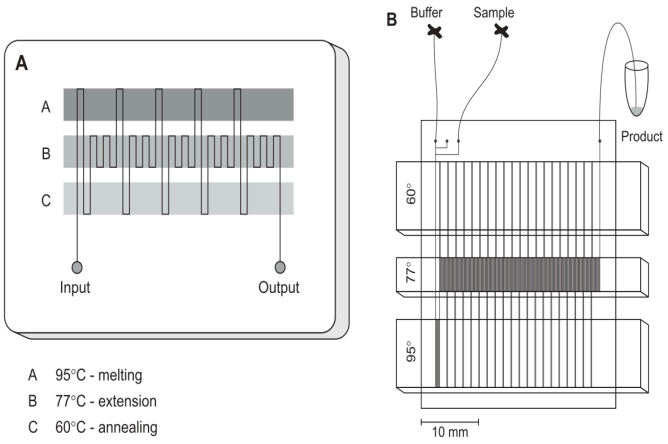

Figure 2. Continuous-flow PCR on chip. (A) Three zones on the chip are maintained at the respective temperatures for each of three stages of a PCR cycle, by copper blocks. (B) The setup used by Kopp et al., consisting of inlets for the sample and buffer and an outlet for the product.[4]

Hanyoup Kim et al. performed PCR in nanodroplets using a low power (~30 mW) infrared laser heating to drive temperature cycling.[7] Their method improves upon past droplet-based PCR chip approaches because the heat source is independent from the PCR mixture. The researchers found optimal conditions of 10 seconds at 93°C for enzyme activation, followed by 40 cycles of 2 seconds at 93°C for melting and 8 seconds at 58°C for annealing/extension. The selective, high-speed heating scheme completes 40 cycles of PCR in 370 seconds.[7] The disadvantage of droplet PCR techniques is that droplet formation is a sensitive and precise process that is prone to human error.[6]

Jinjoo Kim et al. developed a light-based PCR, photo-PCR, for detection of bacteria. Integrating bacterial cell lysis and DNA amplification into a single step significantly reduced overall thermal cycling time. The method utilizes the high heat conductivity of gold nanoparticles (GNPs), through the addition of poly(ethylene glycol)-modified gold nanorods (PEG-GNRs) to the PCR mixture

as a heat generator. GNRs possess superior near-infrared absorption and photothermal conversion properties compared to other gold nanomaterials. The PEG-GNRs are irradiated with an 808-nm laser for rapid and stable thermal cycling between 50°C and 85°C. The researchers identified a range of 50-85°C, PEG-GNR concentration of 0.24 nM and pre-heating of 80-85°C to be optimal conditions for photo-PCR. Photo-PCR reduced overall reaction time to 38 minutes, a significant decrease from the over 1 hour time for conventional PCR.[1]

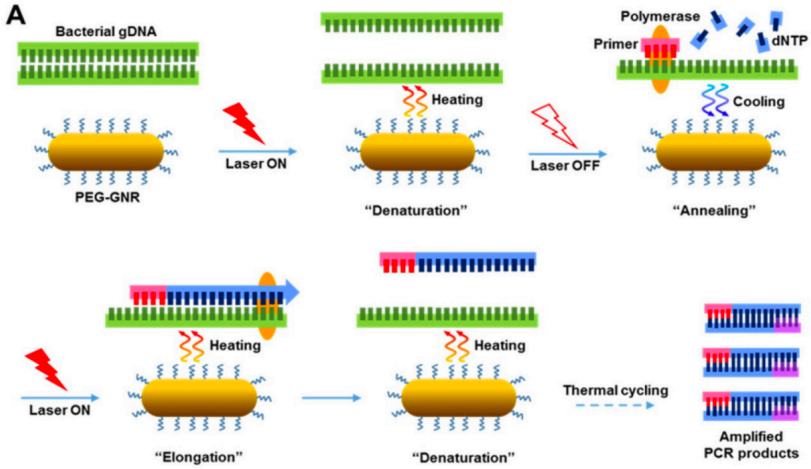

Figure 3. Photo-PCR with GNRs. Laser pulses heat up gold nanorods in solution and drive thermal cycling for PCR. [1]

1.3 Gold Nanorod Heating

Gold nanorods exhibit high photothermal conversion efficiency, making them attractive for use in PCR thermal cycling in a microfluidic environment.[8] GNRs have high absorption cross-sections in the visible and near infrared (NIR) range. Their longitudinal surface plasmon resonance can convert NIR energy into thermal energy.[9]

Kwang Ho Cheong et al. studied the transformation of infrared energy to thermal energy by GNRs in a microfluidic chip. The source of excitation energy was an 808 nm infrared laser. It was found that GNPs with rod-shape have higher optothermal efficiency than those with spherical bead shape. After laser irradiation, spherical nanoparticles demonstrated weaker temperature-increase than nanorods did. This shows that GNRs have an advantage in PCR efficiency due to their heat transfer properties.[9]

Zhiyong Li et al. reported a heating method for droplets in microfluidic chips. Their method uses an 808 nm laser to heat GNRs contained in the laser. Localized heating of individual droplets containing GNRs have the characteristics of rapid response, large temperature range, and precise temperature control. It was found that the temperature of the droplet ranged from 22 – 95°C and was linearly proportional to the laser power. Thus, PCR thermal cycling is possible by controlling the laser power and heating times. The total time for one cycle is only 75 seconds, due to 1-second transitions between steps.[8]

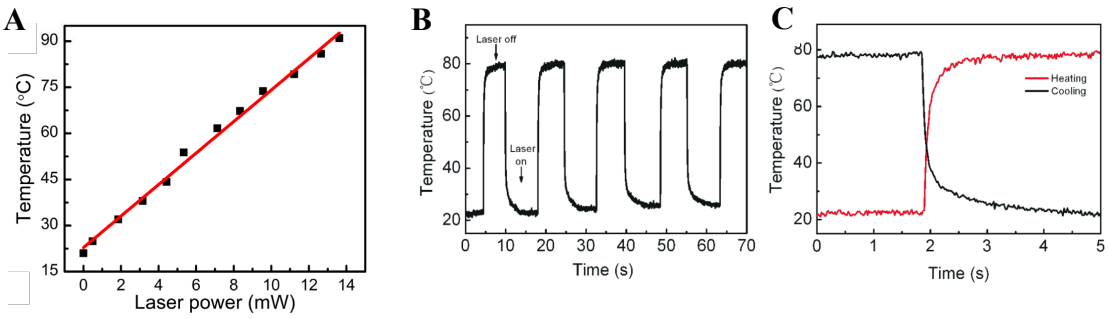

Figure 4. GNR laser-powered heating dynamics. (A) The temperature of a droplet containing gold nanorods increases linearly with laser power. (B) Droplet temperature rises and falls when an 11 mW laser is turned on and off, respectively. (C) Heating and cooling rates are time-dependent; heating takes ~200 ms and cooling takes ~800 ms.[8]

Son C. Nguyen et al. studied the heat transfer dynamics from GNRs to the local water environment. The heat localization and temperature increase of the surrounding environment depends on the rate, the amount of heat transferred, and the heat diffusion from the local solvent to the bulk. They find that longer laser pulse results in a larger local heating region and stronger laser power linearly increases the temperature increase. Furthermore, after 2 ns, most heat is transferred from the nanoparticles to within 34 nm surrounding solution and heat transfer times are generally independent of laser excitation power.[10]

O. Ekici et al. presented a computational model to analyze the heating of GNRs by femtosecond laser pulses of 80 MHz repetition rate. The model shows that water temperature at the nanoparticle surface reaches ~90% of critical value at the fluence of 4.70 J/m^2 and the melting temperature of 1337 K at the fluence of 5.10 J/m^2.[11]

While much research has been conducted on the use of laser excitation for plasmonic photothermal heating of GNRs, lasers are expensive, making this technique not particularly suitable for point-of-care (POC) devices. Jun Ho Son et al. presented a novel photonic light-to-heat conversion method using light-emitting diodes (LEDs), illustrated in Figure 5. Using a thin gold film with 65% optical absorption at 450 nm, ultrafast DNA amplification was successfully achieved in 5 minutes. The LED is capable of heating the gold film and surrounding solution to the necessary PCR temperatures of 55°C to 95°C, up to a maximum of 150°C. The researchers found that light-to-heat conversion

efficiency increases with gold film thickness and is greatest for the blue LED with a 450 nm peak wavelength.[6]

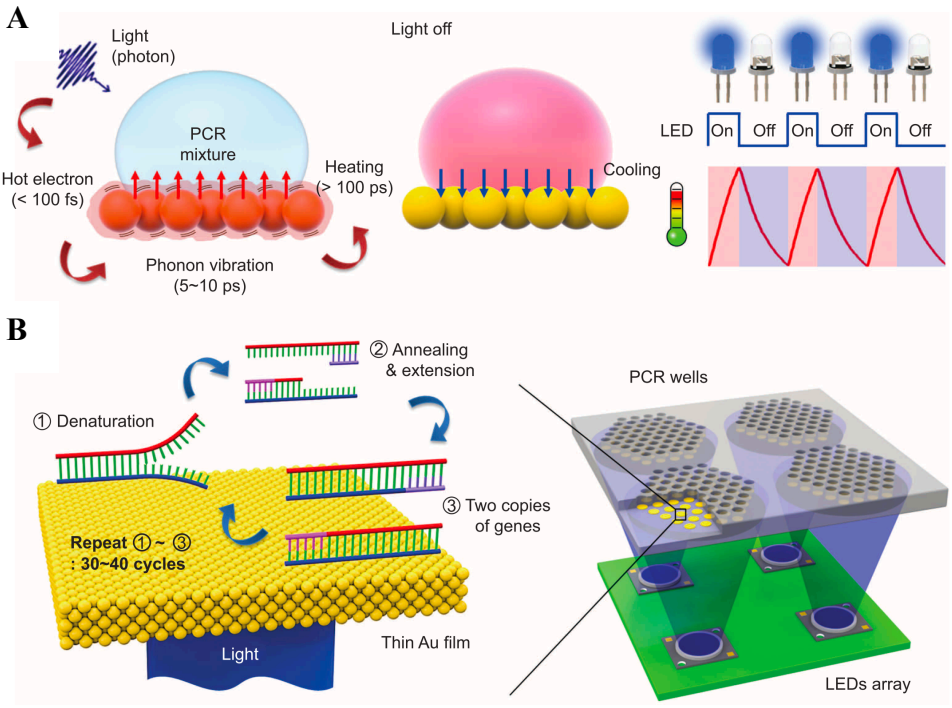

Figure 5. LED-powered PCR with gold nanorods. (A) Gold nanoparticles convert light energy from the LED into heat, which heats up the surrounding solution. Heat dissipates and the solution cools when the LED is off. (B) The gold film facilitates ultrafast PCR cycles for DNA amplification. Several reactions of PCR can be carried out simultaneously, with different temperatures for different PCR primers [6]

1.4 Prior Work Using Gold Nanorods for Biosesnsing

The Zhang lab has previously conducted extensive research regarding the use of gold nanorods for detection of circulating tumor DNA (ctDNA). The group has focused its work on pancreatic ductal adenocarcinoma, and specifically the G12V mutation in the KRAS gene. Pancreatic ductal adenocarcinoma has a high death rate and specifically the G12V KRAS mutation is present in 37% of pancreatic cancers. [12–14]

The combination of gold nanorods conjugated to specific peptide nucleic acid (PNA) probes is able to detect point mutations in ctDNA, without any DNA amplification or labeling. The advantages of such a liquid biopsy technique include minimal invasiveness, low cost, and short time between data collection and result. These characteristics make it attractive to point-of-care applications. The Zhang lab previously experimented with various concentrations, sonication times, and incubation times to optimize the gold nanorod – PNA surface conjugation protocol for sensing and efficiency. The nanorods have dimensions of 40 x 124 nm. The group has also determined the optimal incubation time and concentration of nanorods in solution for ctDNA sensing. Conjugation and biosensing success are determined by measuring the absorbance of the gold nanorods. The baseline absorbance of the unconjugated gold nanorods exhibits a strong peak at 780 nm. After conjugation with the PNA, the peak flattens significantly and shifts to ~900 nm. Upon incubation and successful binding with ctDNA, the absorbance of conjugated gold nanorods shifts back to around ~800 nm. The group has successfully demonstrated the ability of the PNA-conjugated gold nanorods to distinguish between G12V mutants and wild type ctDNA in buffer as well in patient serum samples. [12]

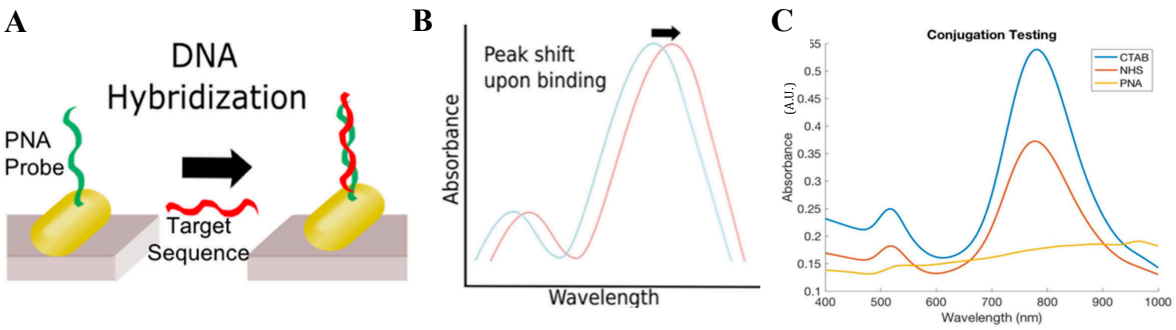

Figure 6. Gold nanorod biosensing and conjugation analysis. (A) CtDNA binds with a complementary PNA probe conjugated to a GNR. (B) The peak absorbance of the 40 x 124 nm GNRs sample shifts to a higher wavelength when ctDNA is bound. (C) The absorbance of the rods shifts to the right and flattens upon successful conjugation (yellow curve). [12]

1.5 Research Contribution

The primary aims of the research are:

(1) Perform surface conjugation to use gold nanorods as selective DNA sensors in solution

(2) Demonstrate light-powered heating of solutions containing gold nanoparticles

(3) Design microchannel for PCR reagent delivery to reaction stages

2. Design and Protocols

2.1 Surface Conjugation for Cancer Mutation Detection

The protocol for conjugation of peptide nucleic acids (PNAs) to gold nanorods was adapted from EDC-NHS chemistry described in several sources. [15–20] To lessen the extent to which nanorods stick to the tubes and become lost, low retention silicon-coated microcentrifuge tubes were used. 20 µl of gold carboxyl functionalized 40 nm nanorods were centrifuged for 8 minutes at 6,000 rpm. After

supernatant removal, 100 µl of 10 mM cetyltrimethylammonium bromide (CTAB solution) was added to the pellet of rods. The rods were resuspended in solution through sonication in ice water and the absorbance of this solution was measured to establish a pre-conjugation baseline spectrum. Next, the nanorod solution was centrifuged for 8 minutes at 6,000 rpm. The supernatant was removed and 1 µl of 375 mM of 1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC), 1 µl of 937.5 mM of N-hydroxysuccinimide (NHS), and 98 µl of Activation buffer were added. Activation buffer is a mixture consisting of 0.1 M MES and 0.5 M NaCl in molecular grade water, at pH 6.0. After a brief sonication in ice water to resuspend the nanorods into solution, the solution was incubated at room temperature for 15 minutes on a rotary shaker mixer to allow for activation of the COOH group with EDC/NHS coupling.

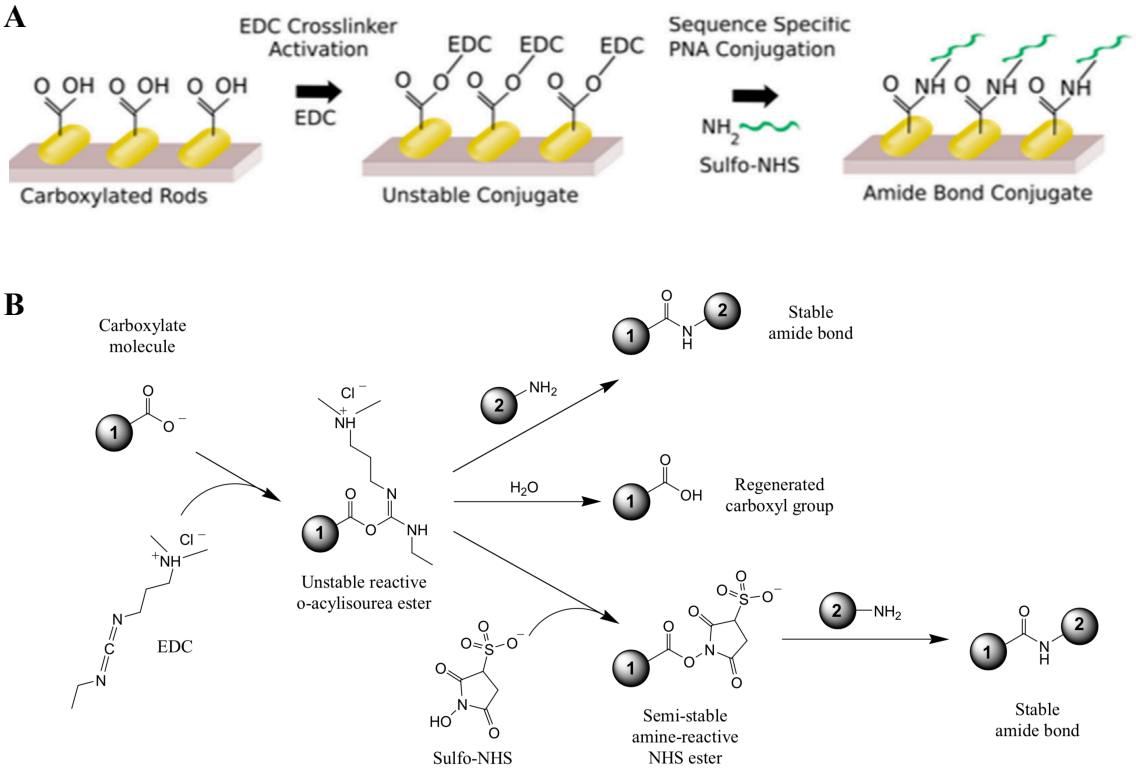

Figure 7. EDC-NHS coupling chemistry. (A) The conjugation of gold nanorods to PNA is achieved through EDC-NHS coupling.[12] (B) In the conjugation of PNA probes to gold nanorods, the group labeled "1" is a GNR and the group labeled "2" is a PNA.[21]

After the incubation, the solution was spun down for another 8 minutes at 6,000 rpm to remove excess NHS and prevent further EDC/NHS coupling. Finally, 2.4 µl of PNA was added, along with 97.6 µl of a combination of Activation buffer and carbonate-bicarbonate buffer in a 5:3 ratio, resulting in an end solution with a pH of ~7. The solution was sonicated to resuspend the nanorods and then incubated at room temperature for 2 hours on the rotating mixer. Afterwards, the nanorods solution was sonicated briefly once again and the absorbance was measured to determine conjugation success. [12,21]

2.2 Microfluidic Design

As aforementioned, microfluidic devices offer many advantages such as rapid heat transfer, reduced reagent consumption, lower cost, and ability to integrate several processes into one workflow. For PCR, miniaturization reduces total analysis time from 1 to 2 hours to under an hour. There are two major types of microfluidic PCR designs: space domain and time domain devices.

Space domain PCR devices move the sample through a microchannel that has fixed temperatures depending on position. For these types of devices, the number of PCR cycles and duration of each step is fixed by the device design. Serpentine channel designs usually consist of three copper heaters at the temperatures for denaturation, extension, and annealing. The channel turns back and forth through the three zones, heating and cooling the sample as it is pumped through. Different variations on the serpentine design have simplified the device and reduced the amount of equipment needed for function. Radial designs improve upon the serpentine design by requiring only one heater, positioned in the center. Annealing and extension zones are created by the natural thermal gradient as the channel moves away from the central heat.

Time domain PCR changes the temperature of the sample, which is stationary, using active heating and cooling. Thus, the speed and number of PCR cycles can be modified by changing the thermal cycling protocols. Many different types of heating have been tested, including tungsten lamps, infrared lasers, and LEDs. These approaches have demonstrated heating rates of 10°C per second, 60°C per second, and 13°C per second, respectively. Cooling can occur passively

or be expedited using a fan. Because samples have a fixed position, they can be printed in arrays

Microfluidic PCR allows for the integration of sample preparation, time domain PCR, and post-PCR analysis. Research has shown the combination of cell lies and DNA extraction, followed by PCR. After PCR, a variety of analyses have been carried out, such as: (1) confirmation of amplification via fluorescence measurements, (2) DNA extraction, real-time detection, quantification and extraction via capillary electrophoresis. [22]

2.3 Heating Calibration

A Horiba Scientific 785 nm laser was used in the initial attempts to heat water droplets, because a significant portion of the existing literature and research on miniaturized PCR incorporating gold nanoparticles used 808 nm lasers as the thermal cycling heat source. The lasers available for experimentation had wavelengths of 785 nm and 515 nm. When these lasers were directed at small water droplets, only minimal heating (temperature increase of 1°C) occurred. This is likely due to the low power of the lasers, as the 785 nm laser is very close in wavelength to the 808 nm lasers used other researchers' experiments. Because of this equipment restriction, an alternate heat source, LED, was identified. The advantages of LEDs over lasers are that they are lower-cost and easier to use.

The temperature change over time of a sample of gold nanoparticles in solution was measured to map the power of the LED to temperature changes. The LED used was a royal blue LED from the Lumileds LUXEON C color line. These

LEDs have a peak wavelength that is between 440 and 460 nm, and a typical radiometric power of 532 mW, making them the highest power LEDs in the product line. The LED was soldered onto a board and attached to 30-gauge wire. A Carclo 20 mm fiber coupling optic was used to focus the light from the LED onto the sample, which was placed directly on the fiber coupling optic. The sample was a 1 μL droplet of optical density 1 (OD 1) gold nanospheres, approximately 10 nm in diameter, with a peak absorbance of 515 nm. The setup of the experiment is shown in Figure 9.

To test the capability of the LED to heat the nanospheres droplet, a range of voltage inputs was used and temperature changes of the droplet were measured. The temperature of the droplet was monitored using an infrared camera for 1 minute, with measurements recorded every 15 seconds. The power source was set to a maximum current of 0.27 amps, and voltage was slowly increased until the LED turned on, at 2.5 volts. Thus, 2.5 volts was used as the starting lowest voltage for heating calibration measurements.

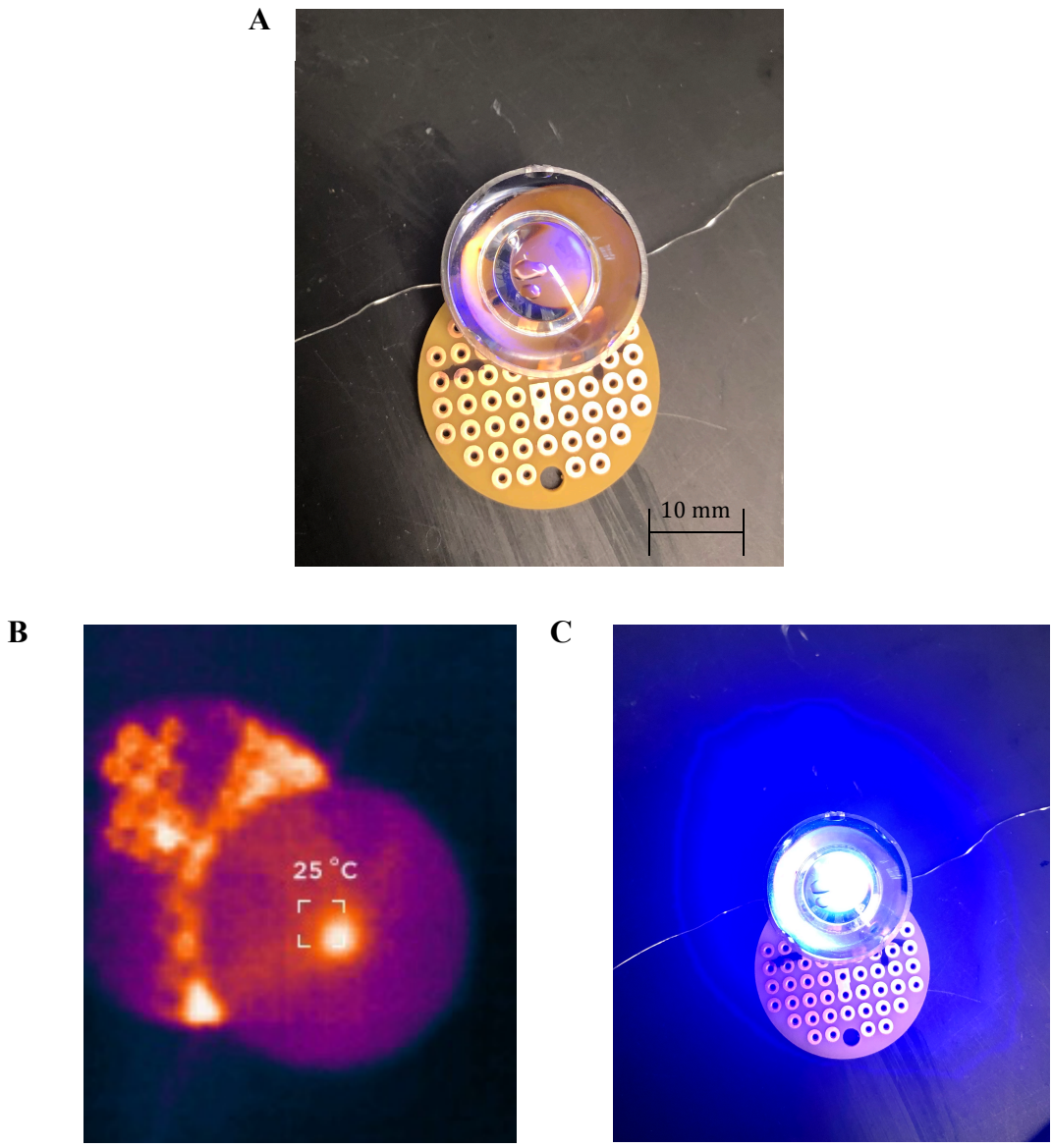

Figure 8. LED heating set-up and testing. (A) The fiber coupling optic was placed over the LED to focus the light. A droplet containing 10 nm diameter gold nanospheres was pipetted directly onto the optic fiber. (B) Measurements were taken with an IR camera. The droplet is the bright spot that the camera is focused on. (C) Increasing the voltage input increases the brightness of the LED.

2.4 Primer Design Towards Integrated qPCR and Sensing

Quantitative real-time PCR (qPCR) allows for the real-time quantification of DNA sequences in a sample. Breitbach et al. demonstrated a method of

sensitive, direct qPCR without the need for DNA isolation or extraction. In other words, DNA of interest can be quantified directly from unpurified plasma, without the need for a DNA purification step. This saves time and lowers the potential for errors or loss of DNA. Two sets of primers were designed using Primer3 software. The primers were designed to amplify 90 bp and 222 bp portions of a consensus sequence. The primers for both amplicons share the same forward sequence. The researchers found that their direct amplification qPCR from plasma resulted in DNA concentrations that were 2.79 times greater than the DNA concentrations obtained via the QIAamp DNA Blood Mini Kit, which includes a DNA isolation step. [23]

Mouliere et al. used an allele-specific qPCR method to analyze and compare concentrations of circulating cell-free DNA (cfDNA). Primers were designed to be specific to circulating cfDNA, using Primer3 and specificity was checked using BLAST. Prior research has showed that greater and more accurate quantification of circulating cfDNA is obtained when the region to be amplified is no longer than 100 bp. Each forward primer was designed to target either the WT sequence or a specific point mutation, allowing for both detection of a specific mutation and determination of the percentage of that mutant allele present in a sample. [24]

Future work in primer design towards qPCR and integrated sensing will utilize strategies similar to the ones summarized here.

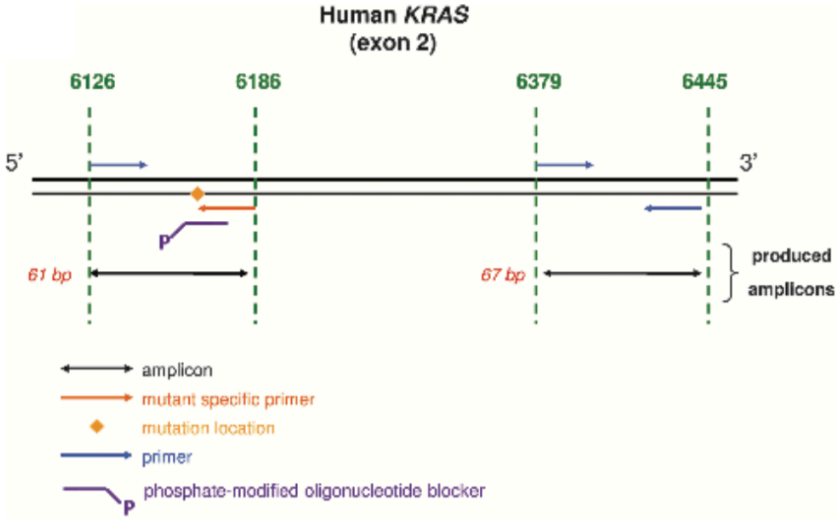

Figure 9. Primer design. Mouliere et al. targeted a KRAS region to quantify concentrations of wildtype and mutant circulating cfDNA. The wildtype sequence is 300bp away from the point mutation. The proportion of mutant allele is calculated as the ratio between mutant concentration and wildtype concentration.[24]

3. Research Outcomes

3.1 Plasmonic Spectra of Conjugation of Gold Nanorods

The protocol for conjugation of PNAs to gold nanorods has been continuously refined such that it reliably yields a successful conjugation when performed. Conjugation success can easily be verified though absorbance measurements. The unconjugated nanorods display a sharp, strong peak at 780 nm. PNA conjugation results in a significantly flatter absorbance curve, with a slight peak around 900 nm.

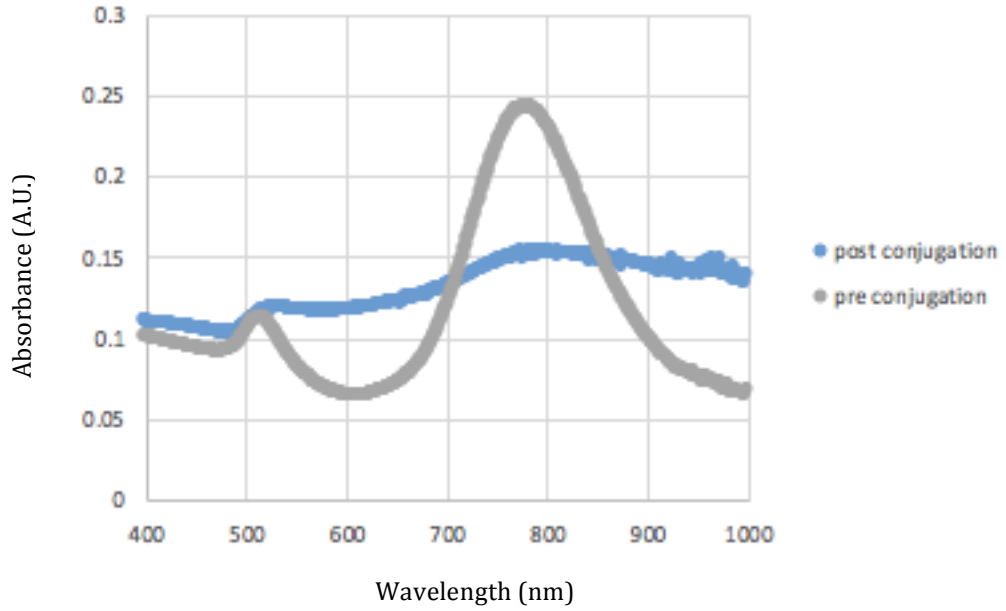

Figure 10. Pre- and post-conjugation absorbance spectra. Upon successful conjugation with the PNA probes, the peak absorbance of the gold nanorods flattens and shifts towards 900 nm.

3.2 Final Microfluidic Design

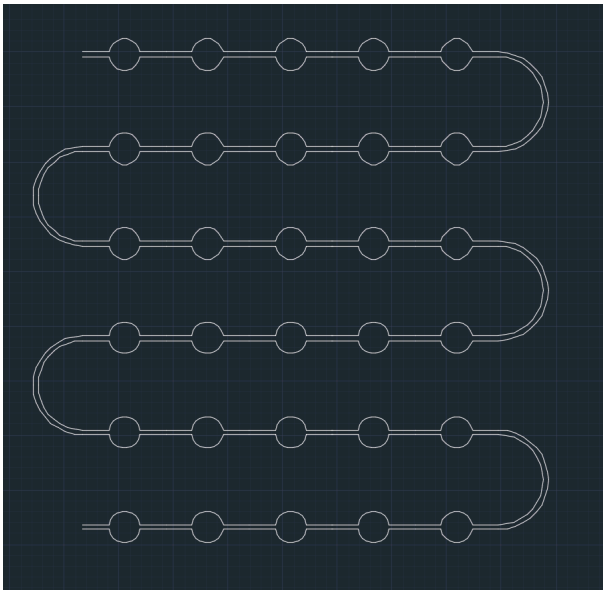

Figure 11. Microfluidic design for PCR on-chip. Serpentine channel with 30 bubbles, one for each PCR cycle.

The microfluidic design is a serpentine channel with 30 bubbles, for each round of PCR. The design fits neither the space domain nor the time domain

methods described previously. In the design, the sample and PCR reagents would be pumped along the channel, and irradiated with a light source at each bubble. The solution would cool as it is pumped towards the next bubble. The number of rounds of PCR carried out is fixed, based on the number of bubbles in the channel.

3.3 Final Heating Table as a Function of LED Input

The results below show the data collected from heating a 1-μL droplet of gold nanospheres with the royal blue LED, tested at a current input of 0.27 amps and voltage inputs ranging from 2.5 to 3.0 volts in 0.1 volt increments. The temperature of the droplet was recorded every 15 seconds during a 1 minute measurement period, for a total of 5 data points for each iteration.

A higher voltage input is correlated with a more rapid increase in temperature, as well as a higher peak temperature reached. At voltage inputs of 2.5 V, 2.6 V, and 2.7 V, droplet temperature stabilized after one minute of heating, at 21 °C for 2.5 V and 2.6 V, and 23 °C for 2.7 V. In contrast, for inputs of 2.8 V, 2.9 V, and 3.0 V, temperatures were still rising after one minute of heating. The droplet receiving a 3.0 V input demonstrated the most rapid rate of heating, with a temperature increase of from 19 °C to 30 °C in 1 minute. While PCR requires a maximum temperature of 80-85°C, this initial heating experiment shows promising results for rapid heating of gold nanoparticles samples by LEDs.

Table 1. LED heating data

	Input Voltage and Resulting Temperature					
Time	**2.5V**	**2.6V**	**2.7V**	**2.8V**	**2.9V**	**3.0V**
0 sec	19 °C	19 °C	19 °C	19 °C	19 °C	19 °C
15 sec	19 °C	20 °C	19 °C	20 °C	21 °C	20 °C
30 sec	20 °C	21 °C	21 °C	21 °C	24 °C	24 °C
45 sec	21 °C	21 °C	23 °C	23 °C	25 °C	26 °C
60 sec	21 °C	21 °C	23 °C	25 °C	26 °C	30 °C

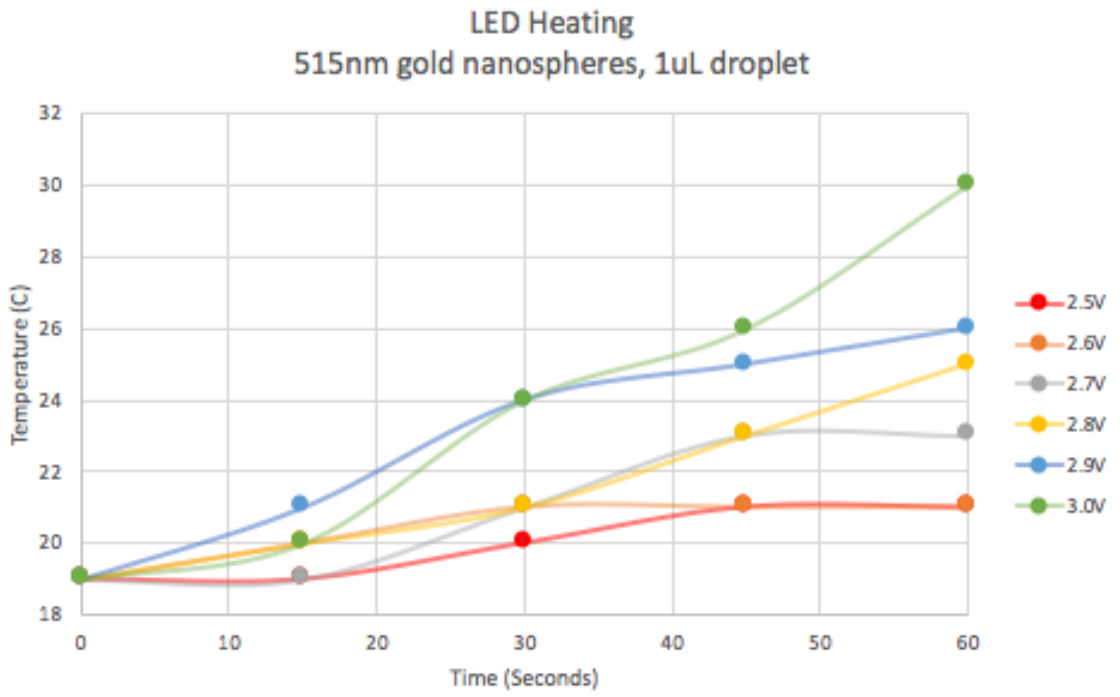

Figure 12. LED heating. The temperature of a droplet of gold nanospheres was measured for 1 minute, while irradiated by a royal blue LED powered by a 0.27 amp current and voltages ranging from 2.5 to 3.0 V.

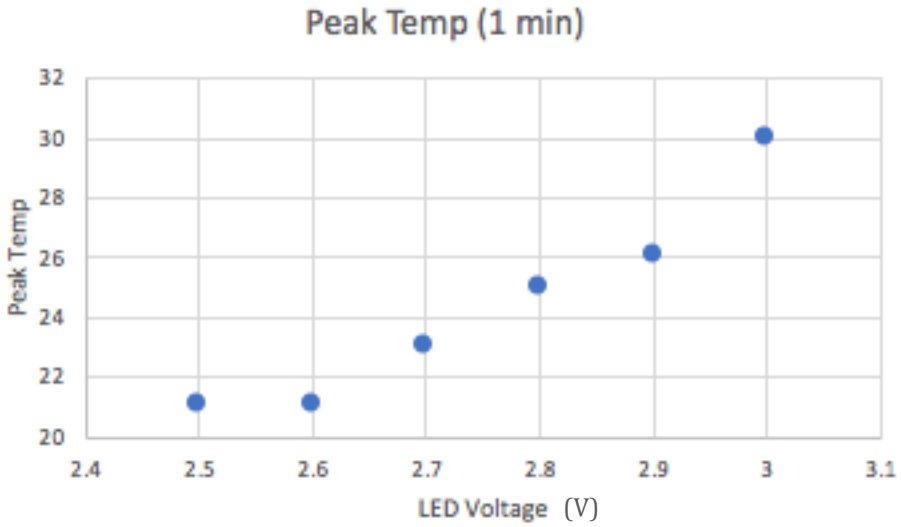

Figure 13. Peak temperatures reached by LED heating. Peak temperature reached by LEDs powered at different voltages, after 1 minute of heating.

4. Outlook & Conclusion

4.1 Applications to Low-Cost Laboratory PCR

While significant research has been conducted on the miniaturization of PCR, it is focused on the use of lasers to drive thermal cycling. The research at hand investigates the use of LEDs instead of lasers as a heat source. Some advantages of LEDs over lasers are lower cost and ease of use. High power lasers are expensive to purchase and have low portability. In contrast, LEDs are small, much simpler to use, and require minimal equipment apart from a power source.

4.2 Applications to Rapid Diagnostics

Liquid biopsy on chip is an increasingly popular technique used in point-of-care devices due to its minimally invasive nature and ability to detect specific biomarkers. Detection can be challenging when samples contain very small concentrations of biomarkers of interest. This problem can be solved by

miniaturized PCR, which is low-cost, easy to use, and consumes small amounts of samples and reagents – ideal for point-of-care applications.

The miniaturization of PCR offers the possibility of combining PCR and detection, allowing for single-step amplification and analysis in one workflow, while also reducing handling time and drastically decreasing the potential for sample contamination. Liquid biopsy is an obvious candidate for post-PCR analysis, as both procedures make use of gold nanoparticles. Gold nanorods are used to facilitate heating, and they can also be used to capture samples of interest for subsequent detection and analysis.

4.3 Future Work

There exists a need to further research and optimize LED heating facilitated by gold nanoparticle. J. Son et al. has successfully demonstrated LED-powered PCR using thin gold film. Future work with regards to the present research will aim to expand the temperature range to at least 80-85°C, the minimum temperature needed for PCR. This can be achieved by testing nanorod geometries and concentrations, pulsed excitation, and LEDs of other wavelengths. Further research will use functionalized conjugated nanorods and include PCR reagents in the droplet to be heated, with the aim of creating an integrated microfluidic PCR and biosensing device.

Appendix A: GNR - PNA Conjugation Protocol

Materials

- EDC Stock (375 mM)
- NHS Stock (937.5 mM)
- PNA
- Au-COOH nanorods
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Buffers

- Activation buffer
    - 0.1 M MES, 0.5 M NaCl, pH 6.0
- Carbonate-bicarbonate buffer
- 10 mM CTAB

Methods

1. Pipette 20 µl of nanorods into a low retention tube
2. Spin down at 6000 rpm for 8 minutes
3. Remove the supernatant and add 100 µl of CTAB
4. Sonicate briefly in ice cold water until resuspended
5. Measure absorbance using 2 µl
6. Spin down at 6000 rpm for 8 minutes
7. Remove the supernatant and add 1 µl of both EDC and NHS stock to the side of the tube
8. Add 98 µl of Activation buffer to the EDC and NHS and sonicate in ice cold water to allow the nanorods to resuspend into solution
9. Incubate for 15 min at RT while mixing
10. Spin down at 6000 rpm for 8 minutes to remove excess NHS
11. Remove the supernatant
12. Add 2.4 µl of the appropriate PNA to the side of the tube
13. Add 97.6 µl of an Activation buffer-bicarbonate buffer mixture with a pH of ~7 (a 5:3 ratio works for pH's of 6.03 and 9.54 respectively)- (61 uL of activation buffer, 37 uL of bicarbonate)
14. Sonicate in ice cold water until resuspended and allow the reaction to proceed for 2 hours at RT while mixing
15. Sonicate in ice cold water after incubation until resuspended and measure the absorbance with 2 µl

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